poisoning by atractyloside, whose mechanism of action resembles that of steviol in some respects, leads to hypoglycemic convulsions[17]. In isolated perfused rat liver, on the other hand, steviol (but not stevioside) also inhibits gluconeogenesis and respiration (Ishii et al., unpublished). This observation proves that the effect of *Stevia rebaudiana* natural products is not restricted to a single tissue. It is thus possible that inhibition of gluconeogenesis plays a significant role in the mechanism of action of *Stevia rebaudiana* natural products.

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Drosophila melanogaster aldehyde dehydrogenase

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Summary. Subcellular fractionation by differential centrifugation confirms the presence of aldehyde dehydrogenase in *D. melanogaster*. It is found principally in the heavy mitochondrial fraction.
Key words. Drosophila melanogaster; aldehyde dehydrogenase; mitochondria.

Drosophila melanogaster is a species frequently found in environments characterized by a high alcohol concentration, and presumably by the presence of some acetaldehyde as well. Both ethanol and acetaldehyde can be dangerous if their concentration is to high, but at lower concentrations they attract the flies and the larvae[3-8]; indeed, both ethanol and acetaldehyde can be used as energy source[8-12]. As it is highly dangerous, the acetaldehyde resulting from oxidation of ethanol by alcohol dehydrogenase (ADH) or catalase must be rapidly metabolized into non toxic products. In most animal species, it is converted into acetate by aldehyde dehydrogenase (ALDH), an NAD^+ dependent enzyme[13-15]. It is commonly assumed that *Drosophilae* do not have any ALDH and that aldehyde oxidase (AO) plays the major role in acetaldehyde conversion[16,17]. Several authors have recently questioned this almost exclusive role attributed to AO[18-20]. Experimental data are in favor of an NAD^+ dependent conversion of acetaldehyde[21-23]. But other authors have a quite different opinion: they consider that 'ADH not only catalyzes the oxidation of ethanol into acetaldehyde, but additionally catalyzes the oxidation of this highly toxic product into acetate'[24]. In an attempt to detect a presumed aldehyde dehydrogenase, and in order to avoid all the difficulties and confusions which could result from the presence of aldehyde oxidase or alcohol dehydrogenase, we chose as our biological material the *bAdh*^{n4} strain (kindly made available by R. Sofer) which lacks ADH as well as AO[24]. It is known that larvae of this strain are attracted by acetaldehyde at low concentrations, as are the larvae of the *Adh*^{n2} strain[7,8].

Flies of the *bAdh*^{n4} strain were submitted to homogenization and to subcellular fractionation by differential centrifugation, according to a method originally used by De Duve et al. for rat liver[25]. Five fractions were isolated. First, a nuclear fraction (N) was separated from a total cytoplasmic extract (E). From the cytoplasmic extract, four fractions were isolated; a heavy mitochondrial fraction (M), a light mitochondrial fraction (L), a microsomal fraction (P), and a final supernatant (S). The activities of nine enzymes were assayed. Of course, our main interest was in aldehyde dehydrogenase, and also in aldehyde oxidase, alcohol dehydrogenase, and catalase. As reference enzymes we used cytochrome *c* oxidase and malate dehydrogenase for mitochondria, acid phosphatase and beta-galactosidase for lysosomes, and NADPH cytochrome *c* reductase for endoplasmic reticulum.

Our main observations can be summarized as follows:

1. The specific activities of reference enzymes, as compared with similar data obtained for rat liver by the same methods, show a much higher value for NADPH cytochrome *c* reductase, a similar value for cytochrome *c* oxidase, and distinctly lower values for lysosomal and peroxisomal enzymes (fig. 1).

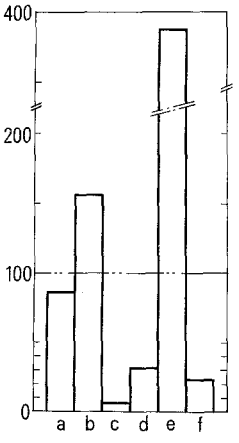

Figure 1. Reference enzymes specific activities: *a* cytochrome *c* oxidase, *b* malate dehydrogenase, *c* acid phosphatase, *d* β-galactosidase, *e* NAPDH cytochrome *c* reductase, *f* catalase. Ordinate: enzymatic activity in *Drosophila* fly, in percent of specific activity for the same enzyme in Rat liver.

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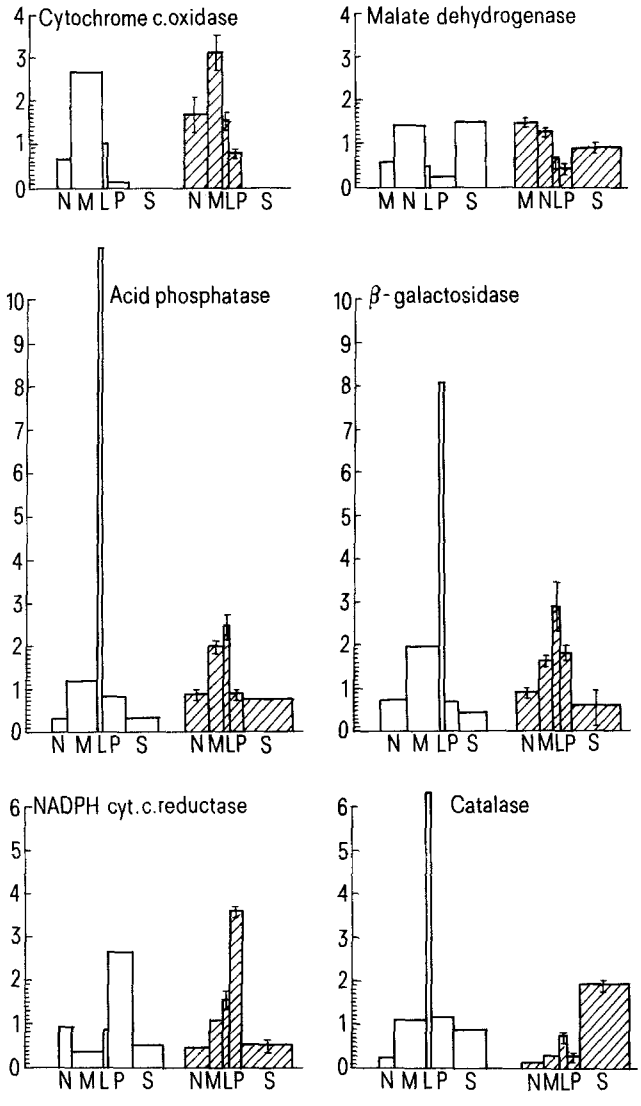

Figure 2. Reference enzymes specific activities in *Drosophila* fly, as compared for each subcellular fraction with the relative specific activity for the same enzyme in Rat liver. Left: Rat liver; right: *Drosophila* fly. N, nuclear fraction. M, heavy mitochondrial fraction. L, light mitochondrial fraction. P, microsomal fraction. S, supernatant.

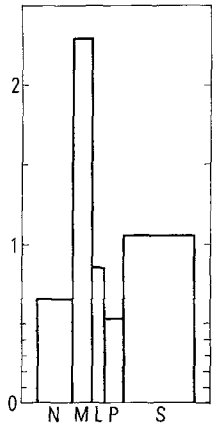

Figure 3. Aldehyde dehydrogenase relative specific activity: distribution pattern in the various subcellular fractions (same designation as in fig. 2).

2. In figure 2 the relative specific activities of the same enzymes are shown, as compared with similar data for rat liver: the distributions in the five fractions are different. A high proportion of cytochrome *c* oxidase is present in the N and the M fractions, but the highest relative specific activity is in the M fraction. Using electron microscopy, it was impossible to discern recognizable nuclei in the nuclear fraction. Malate dehydrogenase is found in N and M fractions and also in the soluble fraction S. NADPH cytochrome *c* reductase is most purified in the microsomal fraction P. About 45% of the lysosomal enzymes are recovered in the M and L fractions. A great difference from rat liver is found for catalase, which plays an important part, in the *bAdh*^{n4} strain, in oxidation of ethanol into acetaldehyde[20]: catalase is mainly unsedimentable. Peroxisomes were not been detected using electron microscopy.

3. As already known, the *bAdh*^{n4} strain lacks aldehyde oxidase and alcohol dehydrogenase.

4. As expected, aldehyde dehydrogenase is present; it is found principally in the M fraction (fig. 3).

Our observations confirm the presence of an active ALDH, as was expected[23]. They are in good agreement with the conclusions of Garcin et al.[21]. Of course, one should not be allowed to conclude that ALDH is the only enzyme, or the most important one, playing a role in the detoxification of acetaldehyde. The respective parts played in this process by ALDH, AO, or even ADH[24], are, besides, probably not the same for the various strains of *D. melanogaster*.

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